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Review article

Up-regulation of VE-cadherin expression by the LMO2 complex promotes vascular remodeling

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Abstract

LMO2 and its binding partner TAL1/SCL have essential roles in both hematopoiesis and angiogenesis. Indeed, LMO2 is highly expressed in endothelial cells and hematopoietic stem cells (HSCs) almost exclusively (with exceptions) throughout life. Recently, the first birthplace of HSCs has been identified as hemogenic endothelial cells in the dorsal aorta in mammals. Firstly, the LMO2 complex including TAL1, GATA1/2/3 (i.e., GATA1 or GATA2 or GATA3), and LDB1 play a key role in endothelial to hemogenic endothelial cell transition in the dorsal aorta in producing HSCs. Another role of the LMO2 complex is to remodel the existing vascular system in angiogenesis. In 2007 and 2012, progress was made in dissecting the mechanism of vascular remodeling regulated by the LMO2 complex. Two of the pivotal role players in vascular remodeling, VE-cadherin and angiopoitin-2, were identified to be among the direct targets of the LMO2 transcription factor complex. In this review, the detailed mechanism in vascular remodeling via the LMO2 complex is discussed.

Key words: LMO2 complex, angiogenesis, VE-cadherin, Angiopoietin-2

Introduction: endothelial phenotype conversion

Remarkable progress has been made in recent years to identify the origin of the first blood cell. Studies have shown that the first hematopoietic stem cells (HSCs) are generated from a hemogenic endothelium intermediate, which is derived from endothelial cells of the dorsal aorta floor in vertebrates. This two-step transition of endothelial cells finally produces HSCs. The first step, transition from endothelium to hemogenic endothelium intermediate, requires a basic-helix-loop-helix (bHLH) transcription factor TAL1, the LMO2 binding partner. On the other hand, the second step, transition from hemogenic endothelium intermediate to HSC, needs the RUNX1 transcription factor (Chen et al., 2009).

Both LMO2 and TAL1 were identified at the breakpoints of reciprocal chromosomal translocations associated with T cell acute lymphoblastic leukemia (Rabbitts, 1994). Gene knockout studies of both *LMO2* and *TAL1* showed a similar bloodless phenotype which is embryonic lethal at around E9.5- E10.5 (Terano et al., 2005). In addition, LMO2 and TAL1 bind to each other to form a transcription factor complex including also E47, LDB1 and GATA1/2/3 (hereafter referred to as the LMO2 complex) (Fig. 1) (Wadman et al., 1997). At present, one of the probable functions (maybe not the first) of the LMO2 complex is to convert "ordinary" endothelium into hemogenic endothelium intermediate (endothelium, which has a potential to differentiate into HSC). Neither LMO2 nor TAL1 is required in the process of vasculogenesis, *de novo* differentiation of endothelium from mesoderm (as discussed in more detail later), because an immature capillary network exists in both Lmo2-/- and Tal1-/- mice. Once HSCs are produced, TAL1 is dispensable for HSC's multipotency, self-renewal, and long-term repopulating activity (Mikkola et al., 2003).

The role of LMO2 and TAL1 in angiogenesis

Our vascular system is constructed by two distinct systems, vasculogenesis and angiogenesis. Vasculogenesis is a process in which a primary capillary network is formed from unspecified mesoderm. Then, a more mature vascular system is made through a process called angiogene-

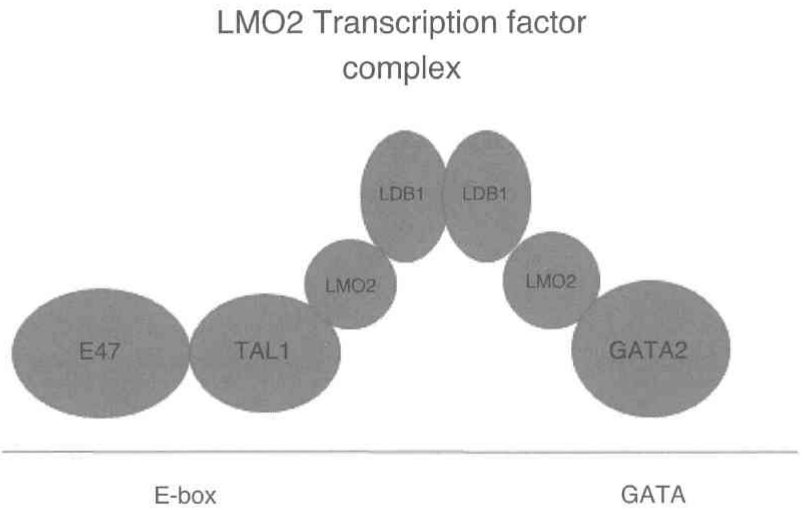

Figure 1 Schematic presentation of the LMO2 transcription factor complex. This complex recognizes the Ebox-GATA sequences on DNA and regulates downstream genes

sis. Angiogenesis involves the remodeling of an existing capillary network to a more complex vascular network. Several years after a bloodless phenotype of Lmo2-/- and Tal1-/- mice was reported, the essential roles of LMO2 and TAL1 in angiogenesis were clarified (Visvader et al., 1998; Yamada et al., 2000). From the present standpoint, the initiation of definitive hematopoiesis is depends totally on angiogenesis because HSCs are produced from endothelium of the dorsal aorta floor. In other words, angiogenesis is prerequisite for definitive hematopoiesis. Therefore, the bloodless phenotype seen in both Lmo2-/- and Tal1-/- mice is now (not at the time of discovery) a foreseeable result of severe defects in angiogenesis due to gene knockout. LMO2 is almost exclusively expressed in endothelium throughout life (Yamada et al., 2000), and TAL1 is also expressed in endothelium. The whole story led us to speculate on the role of the LMO2 complex in angiogenesis. In 2007, Deleuze et al. reported very important results about this (Deleuze et al., 2007).

The LMO2 complex up-regulates VE-cadherin

VE-cadherin (vascular endothelial cadherin) mediates adhesion between endothelial cells and plays an important role in vascular morphogenesis. It is a classical cadherin from the cadherin superfamily and a calcium-dependent cell-cell adhesion glycoprotein composed of five extracellular cadherin repeats, a transmembrane region, and a highly conserved cytoplasmic tail. Although it is dispensable for vasculogenesis, it is essential for angiogenesis. *VE-cadherin* knockout in mice resulted in embryonic lethality at E9.5 due to lack of proper vascular development (Carmeliet et al., 1999). Nascent vessels collapsed or disassembled in the absence of VE-cadherin. VE-cadherin is also known to be required for maintaining a restrictive endothelial barrier and control endothelial permeability. Deleuze et al. reported that the LMO2 complex up-regulates VE-cadherin expression in endothelial cells. First, they performed a TAL1 knockdown experiment using small interfering RNA (siRNA) *in vitro* and found that TAL1 knockdown disrupts endothelial morphogenesis in 2D Matrigel or 3D Collagen I gel HUVEC culture without affecting cell survival. The abnormal intercellular contacts observed in both 2D and 3D networks formed with endothelial cells (ECs) lacking TAL1. This result suggested that the important cell-cell adhesion molecules were down-regulated in TAL1 lacking ECs. As mentioned above, VE-cadherin is one of the strong candidates for such molecules and, in fact, VE-cadherin expression was significantly down-regulated in TAL1 lacking ECs. Secondly, they found that knockdown of E47 and LMO2, both TAL1 binding partners, also down-regulated VE-cadherin expression. Because E47, TAL1, LMO2, LDB1, and GATA2 form a transcription factor complex, this strongly indicated a transcription factor complex composed of E47, TAL1, LMO2, LDB1, and GATA2 (the LMO2 complex) up-regulated VE-cadherin during angiogenesis. Chromatin immunoprecipitation (ChIP) assay using the *VE-cadherin* promoter sequence confirmed that TAL1, E47, LMO2, and GATA2 were

actually recruited to the *VE-cadherin* promoter, and *VE-cadherin* promoter activity depended on a specialized Ebox-GATA element which is an essential sequence for the LMO2 complex binding. This result as a whole shows that the LMO2 complex is a direct upstream transcription factor complex of VE-cadherin.

VEGF signal and VE-cadherin

Homophilic interactions between VE-cadherin molecules at adherens junctions in adjacent endothelial cells help maintain the endothelial barrier. Upon vasculo-endothelial growth factor (VEGF) exposure, activation of VEGF Receptor2 (R2), also known as FLK-1, triggers a phosphorylation cascade that targets VE-cadherin which results in phosphorylation of Y685 by src and S665 by PAK. VEGF R2 contributes to signal after internalization, while sequestration of VE-cadherin in endosomes disrupts adhesion. This process promotes loss of cell-cell contacts, increases vascular permeability, and endothelial cell migration. In this mechanism via VE-cadherin, the VEGF signal promotes vascular remodeling (angiogenesis).

Angiogenesis through angiopoietin/Tie-2 axis

The Tie receptors and their angiopoietin (Ang) ligands have been identified as the second vascular tissue-specific receptor Tyr kinase system. Ang-Tie signaling is essential during embryonic vessel assembly and maturation, and it functions as a key regulator of adult vascular homeostasis. Ang-1- and Tie-2- deficient mice have largely complementary mid-gestational lethal phenotypes, resulting from defects in vascular remodeling and vessel maturation (Sato et al., 1995; Suri et al., 1996). Ang-2-deficient mice have only mild blood vascular defects (Gale et al., 2002). In contrast, global overexpression of Ang-2 causes a phenotype that is reminiscent of Ang-1 or Tie-2 deficiency. These genetic studies have established Ang-1 as the nonredundunt agonistic Tie-2 ligand, whereas Ang-2 is considered to be the antagonist of Ang-1/Tie-2 signaling. This concept has more recently been supported by genetic and biochemical studies establishing a role for endothelium-derived Ang-2 as a negative regulator of Tie-2 phosphorylation. Ang-2 primes and activates endothelium to respond to other angiogenic factors and destabilizes vessel coverage by pericytes, an essential step in initiating angiogenesis.

Angiopoietin-2: Another direct transcriptional target of LMO2 complex

LYL1 belongs to the class II basic helix-loop-helix transcription family and its basic helix-loop-helix sequence shows remarkable similarity with that of TAL1. LYL1 also forms a complex with LMO2 and could thus, be another member of the LMO2 transcription factor complex. Deleuze et al. also showed that LMO2 played a central role in assembling TAL1-E47, LYL1-LYL1 or/and LYL1-TAL1 dimers with GATA2. The resulting LMO2 complex recognizes a highly conserved Ebox-GATA composite element, which lies in the *Ang-2* promoter and up-regulates its expression (Deleuze et al., 2012).

The LMO2 complex binding site is highly conserved in the promoter region of angiogenesis-related transcription factors

Since the LMO2 complex has been identified as a master transcription factor complex for hematopoiesis, which plays a critical role in the emergence of hematopoietic stem cells, the search for the LMO2 complex binding site composed of Ebox-GATA composite elements among the promoter sequences of hematopoiesis-related genes has been carried out.

Now that the LMO2 complex role in angiogenesis has been revealed and members of the LMO2 transcription factor complex have been found in promoter regions of important angiogenesis players such as VE-cadherin and Ang-2, a similar search in promoter regions of angiogenesis-related genes has begun. Interestingly, the analysis of the promoter sequences coming from known angiogenesis-related factors using the MATINSPECTOR program in the TRANSFAC database showed that the binding site of the LMO2 complex is highly conserved in the promoter regions of these factors (Lee et al., 2003).

Transcriptional modulation of endothelial cell development

The transcription factors that regulate endothelial cell development have been a focus of active research for several years, and many players in the endothelial transcriptional program have been identified (Table1). They include members of the Sox, Ets, Forkhead, GATA, and Kruppel-like families. Some, such as the Ets transcription factor Etv2, are involved in the process of vasculogenesis and hematopoiesis, and

Table 1 Knockout mice phenotype of the LMO2 complex related genes

Gene	Lethality	Phenotype	Reference
LMO2 complex			
Lmo2	E9.5~E10.5	Defect in both primitive and definitive hematopoesis. Defect in angiogenesis.	(Yamada et al., 1998) (Yamada et al., 2000)
Tal1	E9.5	Defect in both primitive and definitive hematopoiesis. Defect in yolk sac angiogenesis.	(Robb et al., 1995) (Visvader et al., 1998)
E2A	Neonatal	B lymphocyte differentiation block. Acute T cell lymphoma.	(Yan et al., 1997)
Ldb1	E9.5	Severe defect in mesoderm specification, including blood island of yolk sac.	(Mukhopadhyay et al., 2003)
Gata2	E10.5	Defect in definitive hematopoiesis.	(Tsai et al., 1994)
Downstream			
VE-cadherin	E9.5	Defect in angiogenesis.	(Carmeliet et al., 1999)
Ang2	No	Defect in postnatal angiogenesis.	(Gale et al., 2002)
Upstream			
Etv2	E9.5	Lack of vasculogenesis.	(Lee et al., 2008)
Fli1	E12.5	Defect in hematopoiesis. Disruption of vessel integrity.	(Spyropoulos et al., 2000)

others are solely involved in angiogenesis. The uniqueness of the LMO2 transcription factor complex is its dispensability in vasculogenesis and its key role in hematopoietic stem cell (HSC) production. LMO2 is also indispensable for blood vessel sprouting (sprouting angiogenesis) (Yamada et al., 2002).

Upstream of the LMO2 complex

Ets transcription factors regulate mesoderm specification of endothelial and hematopoietic lineages

The E-twenty-six (ETS) family is a large group of proteins (named after its homology with v-ets oncogene in the E26 retrovirus), with close to thirty members in human and mouse, that achieves transcriptional regulation by binding clusters of ETS binding motifs on gene enhancers and promoters. This conserved core DNA sequence is 5'-GGA(A/T)-3', and a lot of endothelial genes have multiple ETS binding sites in their enhancer or promoter. Hemangioblasts, common precursors of endothelial and hematopoietic cells, are specified from ventral mesoderm during the process of vasculogenesis. Fli1 and Etv2 are members of Ets transcription factors and have recently been identified as master transcription factors for vasculogenesis. LMO2 complex members such as LMO2, TAL1, and GATA2, are upregulated by these two Ets transcription factors.

Etv2: a transcription factor which triggers vasculogenesis and LMO2 expression

During embryogenesis, the endothelial and the primitive erythropoietic lineages first appear during gastrulation in the blood islands of the yolk sac. *Etv2* expression is present at the very early stages of vascular development in the mouse, with expression detected in the blood islands of the yolk sac, and in the earliest vessels in the embryo. Notably, *Etv2* expression begins decreasing within endothelial cell populations by E9.5 and is essentially extinguished in those lineages by E10.5, suggesting an involvement of this transcription factor in early vascular development. *Etv2* null mice have severe defects in vasculogenesis and primitive hematopoiesis. *Etv2-/-* embryos die at midgestation and lack any detectable embryonic vessels, blood islands in the yolk sac, or endothelial progenitors (Ferdous et al., 2009; Lee et al., 2008). Expression of early vascular markers such as Flk1, PECAM, and Tie2, is almost completely abolished in the absence of Etv2. Hemangioblasts are apparently not differentiated from ventral mesoderm without Etv2. Interestingly, Etv2 binds to the LMO2 enhancer and transactivates its expression (Koyano-Nakagawa et al., 2012). LMO2 plays a critical role in primitive red blood cell differentiation in yolk sac blood islands soon after hemangioblasts specification.

Vasculogenesis, angiogenesis, and hematopoiesis

In primitive hematopoiesis (yolk sac erythropoiesis), the blood islands produce the first vascular progenitors in the yolk sac of mammals. Simultaneously, the primitive red blood cells are born in the inner surface of the islands. The LMO2 complex, as well as Fli1, are necessary for this primitive red cell specification. We still don't know when this hematopoietic lineage specifica-

tion occurs. Most likely, blood islands are composed of bi-potent hemangioblasts, which have the potential to differentiate into both hematopoietic and endothelial cells. After the production of the primitive red blood cells, the vascular progenitors form a vascular plexus, which is then remodeled into more mature blood vessels through the process of angiogenesis. TAL1 is essential for this angiogenic process (Visvader et al., 1998).

In definitive hematopoiesis, which is intra-embryonic and produces the pluripotent hematopoietic stem cells (HSCs), the endothelial floor of the dorsal aorta is the birthplace of HSCs. Because the dorsal aorta is constructed by the process of angiogenesis, it is prerequisite for definitive hematopoiesis. As mentioned before, the LMO2 complex is essential for this angiogensis and for the transitional process of the aortic endothelium into hemogenic endothelium. Without the LMO2 complex, no HSCs are produced from the dorsal aorta. On the other hand, the HSCs produced are likely to play some roles in following angiogenesis through Ang1/Tie2 axis. Further research is needed to clarify the interdependence of hematopoiesis and angiogenesis.

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